Bull. Environ. Contam. Toxicol. (2000) 64:347-353

DOI: 10.1007/s001280000006

Bulletin of Environmental Contamination and Toxicology

Organochlorine Compounds in Florida Feral Pigs *(Sus scofa)*

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Received: 22 September 1999

Domestic pigs were introduced to Florida in 1539 by Hernando DeSoto (Towne and Wentworth 1950). The original escapes from the Spanish have since been augmented by escapes from domestic herds (Wood and Barrett 1979). These pigs are a nuisance in public parks and private lands where their rooting behavior destroys landscaping (Howe et al 1981). Feral pigs pass brucellosis *(Brucella suis),* a venereal disease of pigs, to domestic pig herds and brucellosis can be passed to humans who contact infected pigs (Blood and Henderson 1971; Becker et al. 1978)

Water, sediment, soil, air, aquatic invertebrates, and fish have been monitored for persistent organochlorine compounds (Connell et al. 1999). The National Contaminant Biomonitoring Program of the U.S. Fish and Wildlife Service monitored freshwater fish, European starlings *(Sturnus vulgaris)* and duck wings for persistent environmental contaminants (Schmitt et al. 1999). Kenyan commercial swine have been surveyed for organochlorine compounds (Kotonya et al. 1994)

The purpose of this study was to determine the organochlorine compound status of Florida feral pigs in a wildlife management area.

MATERIALS AND METHODS

This study was conducted in central Florida at the Lake Panosoffkee area (Hanover) located in Sumter County Florida. Lake Panosoffkee is the southwestern boundary and I-75 is the eastern boundary of the study area. The dominant habitat is mixed hardwood wetlands primarily in the southern portion. Uplands occur in the northern portion of the tract and include low flatwoods, oak scrub and xeric oak hammock. A large portion of the uplands have been converted to improved pasture. The area contains 3,865 hectares. This area was purchased by the Southwest Florida Water Management District in 1992 and was a private horse ranch and quail hunting preserve prior to 1992.

Before traps were installed, feral pigs were habituated to whole corn by stringing the bait down a selected woods trail over a nine mile transect. Corn feeding stations

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were placed one half mile apart. Traps were placed at each feeding station with trap doors wired open after a 5 day prebaiting period.

Trap dimensions were 8' x 4' x 5' and were constructed of galvanized pipe and bull fencing. The traps door was also galvanized pipe and opened inward. The door was kept open with a treadle and rope and the pigs sprung the trap door with their feeding activity. Only animals 30 pounds and heavier were bled. Trapped pigs were restrained with two locking snout snares of 1/8" aircraft cable by two persons.

In a simultaneous operation, pigs were bled with an 18 gauge needle and 15 mL plastic syringe from the jugular vein, inoculated with RB-51 vaccine, tagged on one ear, notched on the other ear, and ticks were collected. The drawn blood was injected into a vacutainer (purple top, EDTA, Becton-Dickinson, Franklin Lakes, NJ). The blood sample was placed on ice, transported to the laboratory at the end of sampling for that day (about 3 hrs), and centrifuged at 4000 x g for 15 minutes on a Sorvall GLC-2 centrifuge (Dupoint Instr., Newtown, CT). The plasma was filtered with an S/P sampler blood serum filter (Baxter, Inc., McGaw Park, IL), placed in a 15 mL screw cap plastic test tube and stored at -40°C until shipment. The samples were shipped as one lot overnight on dry ice. At the laboratory, samples were processed and analyzed according to Brock et al. (1996). The analytes in this study were: a-chlordane, aldrin, ß-hexachlorocyclohexane, dieldrin, δ-chlordane, δ–hexachlorocyclohexane, heptachlor epoxide, heptachlor, oxychlordane, PCBs 54, 56, 66, 74, 99, 101, 146, 153, 170, 172, 177, 178, 187, 193, 194, 195, 206, o,p-DDT, p,p'-DDT, p,p'-DDE, and trans-nonachlor.

All reagents were analytical grade unless otherwise noted. Water and methanol were HPLC grade. Pesticides were obtained from the U.S. Environmental Protection Agency Pesticides Repository (Research Triangle Park, NC). All PCB congeners will be referenced using a standard numbering scheme (Ballschmitter and Zell 1980). PCBs were obtained from Ultra Scientific Inc. (North Kingston, RI), AccuStandard Inc. (New Haven, CT), or from Cambridge Isotope Labs (Woburn, MA). BZ#l89 was synthesized in an academic laboratory by Stephen Safe. 2,2,4-trimethylpentane (Mallinckrodt, Paris, KY, Nanograde), water (Fisher, Fair Lawn, NJ, HPLC grade), paraffin oil (Fisher, Fair Lawn, NJ), and dodecane (Aldrich, Milwaukee, WI) were all obtained commercially. All glassware was washed with soap and water then rinsed with acetone followed by hexane.

The quality control material was bovine serum previously spiked with three levels of analytes (Burse et al. 1990; Gibco BRL Calf Serum, Life Technologies Inc., Grand Island, NY). One quality control sample and one reagent blank were analyzed with every batch of ten pig samples. All results from the QC materials were compared to limits established in the NCEH laboratory. If the QC materials were out of control limits or demonstrated a trend, the pig samples in that batch were reanalyzed.

All pig samples were analyzed blind. Pig serum samples were spiked with 50 µL of the spiking solution and sonicated for 5 min. All spiked sera were allowed to equilibrate overnight in the refrigerator prior to extraction.

The surrogate spiking solution was prepared by combining stock standards (~10 ppm) of nine compounds in 2,2,4-trimethylpentane and diluting with methanol to a total volume of 100 mL. These nine surrogate compounds are BZ# 18, 30, 42, 65, 116, 204, Endosulfan I, α-HCCH and 2,2',4,4',5,5' hexabromobiphenyl.

A 2 mL aliquot of serum was dispensed into a clean test tube. Two mL of water were added and mixed. Then one mL of formic acid (Kodak, Rochester, NY, 96%) was added to denature the proteins. This mixture was sonicated for 5 min at room temperature. Serum samples were immediately extracted.

A solid phase extraction column, octyldodecyl (SPE-C_{18}) column (J. T. Baker, Phillipsburg, NJ, 7020-33) was pretreated with 10 mL each of: 2,2,4-trimethylpentane, methanol and then water. Each solvent was pulled through the columnusing a vacuum manifold (Supleco Inc., Bellafonte, PA) at -0.5" Hg. Enough water was left on the column to keep the packing material wet. A SPE-Florisil column (J. T. Baker, Phillipsburg, NJ) was pretreated with 15% ethyl ether in petroleum ether followed by 2,2,4-trimethylpentane. Each solvent was allowed to flow freely through the column. Enough 2,2,4-trimethylpentane was left on the column to keep the packing material wetted.

The sonicated mixture of sera, water, and formic acid was added to the top of the prepared SPE-C,, column. Using the manifold, vacuum was applied at -0.5” Hg. Vacuum was maintained until the top of the sample sera was even with the head of the column. The sera was allowed to stand on the column for 5 min. After the 5 min delay, vacuum was resumed. A rinse of 5 mL of water was added to the column and pulled through. Vacuum was maintained on the column until no liquid remained. Then, 10 mL of 2,2,4-trimethylpentane was added to elute the analytes and surrogates. The 2,2,4-trimethylpentane was pulled completely through the column at -0.5” Hg and collected in a clean dry glass tube.

The 2,2,4-trimethylpentane extract from the SPE-C_{18} step was passed through the prepared SPE-Florisil column. Then, 5 mL of 15% ethyl ether in petroleum ether were added to elute some pesticides. The 2,2,4-trimethylpentane and ether extracts were collected in a clean dry tared glass tubes. Samples were then evaporated to 0.5-0.9 mL and 0.1 mL of 125 ppb dichloronaphthalene was added as an internal standard for the GC analysis.

A 2 µL aliquot of each GC sample was injected onto two separate GC’s containing two different columns, a DB5 (30 m x 0.32 mm ID, 0.5 µ film. thickness) and a DB1701 (30 m x 0.32 mm ID, 0.5 µ film. thickness) (J&W Scientific, Folsom, CA). Injector port and detector temperatures were 270°C and 340°C respectively.

Helium carrier gas flow was constant at 1.7 mL/min. The make-up gas for the ECD detector was 5% methane in argon at a flow of 40 mL/min. The temperature programs for both columns were identical. The initial temperature of the columns was 90 °C which was held for 4 min. Then, the temperature was increased to180°C at a rate of 18°C/min and was held for 1 min. Then, the temperature was increased to 200°C at a rate of 1 °C/min and held for 1 min. Finally, the temperature was increased to 275 °C at a rate of 1.5 °C/min and held for 5 min.

Elution times were determined by injecting a standard of each analyte separately. Only completely resolved peaks for each analyte were used for quantification. Quantification was accomplished by comparing peak area ratios (analyte/internal standard) to a linear calibration curve. The curve was generated by analysis of standards at six different concentrations in triplicate. Recoveries of compounds ranged from about 50% (heptachlor) to about 72% (p,p'-DDD) with SEM from about 1 to 4 (Brock et al. 1996).

Serum specimens were analyzed for cholesterol and triglycerides using standard enzymatic methods. Total lipids were calculated from total triglycerides and total cholesterol using a standard formula (Phillips et al. 1989).

We caution other researchers that this work is dangerous. Brucellosis is known as undulant fever in humans. Three of the authors have had this disease from experiments with feral swine. Researchers should avoid contact with tissues, blood, urine and aborted fetuses from infected animals. Also, these are wild animals and a 200 lb wild swine can easily injure or kill a human.

RESULTS AND DISCUSSION

One female feral pig had 0.63 ppb p,p'-DDE in its blood plasma (Table 1). One male had 0.15 ppb PCB-74 in its blood plasma. Six of 12 females had 0.16-0.19 hexachlorobenzene (HCB) in their blood plasma. For feral males HCB ranged from 0.15-0.22 ppb in six of 11 animals. Two domestic pig plasma samples contained 0.64 and 0.67 ppb, p,p'-DDE (Table 1). Analytes not reported were below the limit of detection.

About 50% of male and female swine had HCB in their blood plasma. This compound is used as a fungicide in grain and appears in the environment as a manufacturing byproduct (Spectrum Laboratory 1999). HCB may be passed from mother to offspring in milk (Nakashima et al. 1998) and is found in humans (Schlumer et al. 1998; Phillips et al. 1989). The toxicology of HCB has been extensively studied (Kuiper-Goodman 1977, Yang et al. 1978).

Our data on HCB may have come from the whole corn we used for bait. Unfortunately, no sample of the bait corn was analyzed. HCB has been found in humans (1-310 ppb), in human milk (1-25 ppb), in various foods, in landfill soil

Table 1. Organochlorine compounds in Florida feral pig serum (ng/mL)

	Sample	Weight (lb)	Description	T	C	Total lipid (g/L)	Compound	Detection limit
1	Female	65	lactating	71	63	215	ND	--
2	Female	65	lactating	88	137	400	0.17 HCB	0.10
3	Female	65	pregnant	95	39	184	0.17 HCB	0.10
6	Female	65	w/piglets, lactating	136	83	325	ND	--
7	Female	65	w/piglets, lactating	133	68	288	ND	--
13	Female	50	adult, normal	113	128	404	0.63 p,p'-DDE	0.20
14	Female	35	adult, normal	67	61	206	0.20 HCB	0.10
17	Female	60	adult, normal	108	102	340	ND	--
410	Female	40	lactating	80	58	212	0.19 HCB	0.10
110	Female	80	lactating	93	78	271	0.16 HCB	0.10
210	Female	75	lactating	102	79	282	0.16 HCB	0.10
310	Female	75	adult, normal	76	72	240	0.16 HCB	0.10
19	Male	80	adult, normal	91	52	210	ND	--
16	Male	130	adult, normal	90	49	202	ND	--
18	Male	80	adult, normal	75	60	212	ND	--
12	Male	50	adult, normal	113	43	211	0.15 PCB-74	0.20
15	Male	140	adult, normal	80	36	162	ND	--
11	Male	170	adult, normal	76	52	195	0.18 HCB	0.10
8	Male	110	adult, normal	63	99	288	0.17 HCB	0.10
9	Male	80	adult, normal	84	55	209	0.20 HCB	0.10
10	Male	140	adult, normal	113	48	223	0.19 HCB	0.10
4	Male	245	adult, normal	101	39	190	0.22 HCB	0.10
5	Male	160	adult, normal	87	52	206	ND	--
U234	3 males, mixed	45, 100, 75	gelts, domestic	123	39	212	0.67 p,p'-DDE	0.20
U167	3 female, mixed	65, 300, 45	normal adult	94	39	183	0.64 p,p'-DDE	0.20

*Calculated. Total lipid = 2.27 (total cholesterol) + triglycerides + 0.623 in g/L (Phillips et al. 1989)
T = Total triglycerides
C = Total cholesterol
ND = Not detected

(5 x 10^6 ppb), in wild boar fat (410 ppb) (Germany), in foxes (Germany), in wild birds, in fish, in shellfish, in fresh and sea water (Courtney 1979). HCB is very persistent in soil (Courtney 1979). Compared to other animals the HCB content in Florida feral pigs was very low. Florida feral pig HCB levels are comparable to U.S. freshwater fish (Schmitt et al. 1999). The mean home range of a boar ranges from 2.08-4.35 Km^2 and for sows 0.81-1.51 Km^2 (Sterner 1977). Within this range and feeding on natural foods persistent organochlorine compounds were either absent or very low in Florida feral pigs. Garbage fed domestic pigs contained p,p'-DDE. This is the first study of persistent organochlorine compounds in Florida feral pigs.

Acknowledgments. We thank Elaine N. Gunter for lipid analyses. Florida Agricultural Experiment Station Journal Series No. R-07116.

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